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Suppression of Food Intake in *Triatoma infestans* by *N*-Substituted Methyl Maleamates

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Abstract: A series of methyl esters of *N*-substituted (*Z*)- and (*E*)-maleamic acids were synthesized and their effect on food intake measured on fifth-instar nymphs of *Triatoma infestans*. Suppression of food intake was found only for the (*Z*)-isomers. The initial reaction rate of the synthesized compounds with glutathione (GSH) was calculated from the reaction *in vitro* of the (*Z*)-isomers. No reaction was observed with the (*E*)-isomers. Good correlation between the suppression of food intake, measured by its ED_{50} (effective dose that inhibited feeding of 50% of the population) and the initial reaction rate with GSH and the hydrophobic parameter π, was found.

Key words: (*Z*)-maleamates, (*E*)-maleamates, suppressed food intake, QSAR, *Triatoma infestans*

1 INTRODUCTION

Previous work in our laboratory demonstrated that *N*-ethylmaleimide (NEM) and other —SH reagents, when topically applied on nymph V and adults of *Triatoma infestans*, Klug (a blood-sucking reduviid bug, vector of Chagas disease), resulted in a suppression of food intake,* attributed to a chemoreceptor blockage produced by —SH reagents.[1]

Almost all the hypotheses at present postulate some important role in chemoreception to —SH groups.[2,3] Some authors have suggested that insect chemoreception is affected by the intracellular concentration of reduced glutathione (GSH).[4]

Our laboratory has recently demonstrated that the GSH content, measured in antennae of nymph V of *T. infestans* as GSH + GSSG, diminishes 70% in insects, with some feeding inhibition produced by NEM. (Fontan A. and Zerba E., unpublished).

* In this paper the notion 'suppression of feeding' is preferred to distinguish it from feeding deterrence, antifeedant effect or feeding inhibitory action. The latter terms, which are often applied in too wide a sense, should be retained in their original narrow meaning when inhibition or cessation of feeding behaviour is the direct and immediate consequence of the host (food) source.

It is known that NEM is one of a number of *N*-substituted maleimides which reacts readily with thiols because of its C=C double bond. It is also known that NEM is not very stable; in aqueous solution the pyrrole ring opens leading to the formation of *N*-ethyl maleamate.[5]

In an attempt to obtain more stable compounds than NEM, but with similar effects on *T. infestans* chemoreception, that would lead finally to a reduction of the population density, the methyl esters of a series of *N*-substituted maleamic acids were synthesized.

In the present study, we examined the suppression of food intake by a series of (*Z*) and (*E*)-isomers of the methyl ester of *N*-substituted maleamic acids (*N*-ethyl, propyl, butyl, hexyl, heptyl and octyl). The extent of the effect correlated with their chemical structure characterized by the hydrophobic parameter π of the *N*-substituent and with their initial reaction rate with GSH.

2 EXPERIMENTAL METHODS

2.1 Chemicals

N-substituted maleamic acids were prepared in very good yield according to Mehta *et al.*[6] and, as previously reported, purified by recrystallization from ether.[7]

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Diazomethane was prepared from Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) (Aldrich) according to a general method.[8]

Ethyl, *n*-propyl, *n*-butyl, *n*-hexyl, *n*-heptyl and *n*-octyl amines were purchased from Aldrich and freshly distilled.

Thionyl chloride was from Aldrich and used freshly distilled. All solvents were analytical grade and kept over 4 Å molecular sieves.

2.2 Synthesis

The structures of all synthesized compounds were supported by their proton magnetic resonance spectra obtained with a Varian EM 360-390 spectrometer in deuterochloroform as solvent. TLC was performed on silica gel plates using toluene + carbon tetrachloride + isopropanol (6 + 1 + 1 by volume).

2.2.1 Synthesis of (Z) N-*substituted methyl maleamates*
To a solution of 0·005 mole of *N*-substituted maleamic acid (*N*-ethyl, propyl, butyl, hexyl, heptyl and octyl) in anhydrous methanol or ether at 0°C, diazomethane in ether was dropped until a yellow colour persisted. The reaction was followed by TLC and stopped when the acid had disappeared, to avoid the formation of by-products. Solvent was evaporated under vacuum and the (*Z*)-isomer of the methyl ester was obtained. The products were recrystallized from hexane + acetone.

2.2.2 Synthesis of (E) N-*substituted methyl maleamates*
To a solution of thionyl chloride (5 ml) in methanol (100 ml) at 0°C, *N*-substituted maleamic acid (0·05 mole) was added, in small portions, with stirring. The temperature was allowed to rise up to 40°C. The methanol was distilled off under vacuum, the residue washed with sodium hydrogen carbonate and extracted twice with ether. After evaporation of the solvent, the remaining oil was distilled under vacuum. The products thus obtained usually crystallized at 0°C giving white solids that were recrystallized from hexane + acetone.

2.3 Biological assays

2.3.1 Biological material
T. infestans were obtained from a colony maintained in our laboratory at 30°C and 50–60% RH.[9] The experimental work was done on 12 to 14-day-old stage V nymphs, starved since last feeding in the preceding instar.

2.3.2 Suppression of food intake
Stage V nymphs were treated by topical application with 1 μl of an acetone solution of the test compound on the ventral surface of the abdomen as previously described.[1] Two duplicate batches of five nymphs were treated with solutions of the compounds at five dose levels. After treatment, each batch was confined in a clean plastic jar. In the control group insects were treated with acetone. An immobilized pigeon as source of blood was offered 24 h later to all insects and their weight increase measured in order to obtain the number of fed insects.

The suppression of food intake after topical application was measured as the dose necessary to inhibit complete feeding behaviour in 50% of the treated insects (ED_{50}). In the unfed insects a null increase of weight was observed while, in the fed insects, a weight increase of at least three times the initial body weight was measured.[1]

2.4 Statistical treatment of results

ED_{50} values for all the synthesized compounds were calculated by a computer program based on the 'probit' method.[10,11]

2.5 Reaction with glutathione

Solutions ($4{\cdot}25 \times 10^{-4}$ M) in dioxane of all the (*Z*)-isomers of the synthesized compounds and the (*E*)-isomers of *N*-hexyl and *N*-octyl methyl maleamate were prepared. The variation of the absorbance with the wavelength was registered for each compound tested on a Shimadzu UV-160 spectrometer, and the λ_{max} registered.

GSH ($4{\cdot}25 \times 10^{-4}$ M) in buffer tris(hydroxymethyl)aminomethane (0·3 M, pH 8·5) was prepared.

One point five millilitres of each solution was mixed, and the initial absorbance was measured. All the reactions were performed in a water bath at 60°C and the decrease of absorbance was registered each 10 min. The reaction mixtures were cooled to 0°C to stop reactions prior to absorbance measurements.

The concentration expressed in millimole of the *N*-substituted methyl maleamates was plotted against the reaction time. The initial reaction rates were obtained by calculating the curve derivative at $t = 0$.

2.6 Density of charge on β-C

The β-C is defined as the double-bonded carbon adjacent to the —CONHR group (Fig. 1).

Molecules were built using a molecular modeling software (PCMODEL 4.0 software, Serena Software, Blumington, IN, USA). Their geometries were optimized using the molecular mechanics force field MMX (MMX is derived from MM2 force field of Allinger,[12] with the *pi*-VESCF routines taken from MMPI, as given by Allinger).

Values for the density of charge corresponded to the proportion of total electrons carried by the β-C. The

TABLE 1
NMR of Characteristic Group

N-*substituted methyl maleamate isomer*	*Chemical shift*		*Coupling constant*
	HC = CH α	*HC = CH β*	*J (Hz)*
E	6·7–6·8	7·0–7·2	15 ~ 16
Z	6·1–6·2	6·4–6·5	12 ~ 14

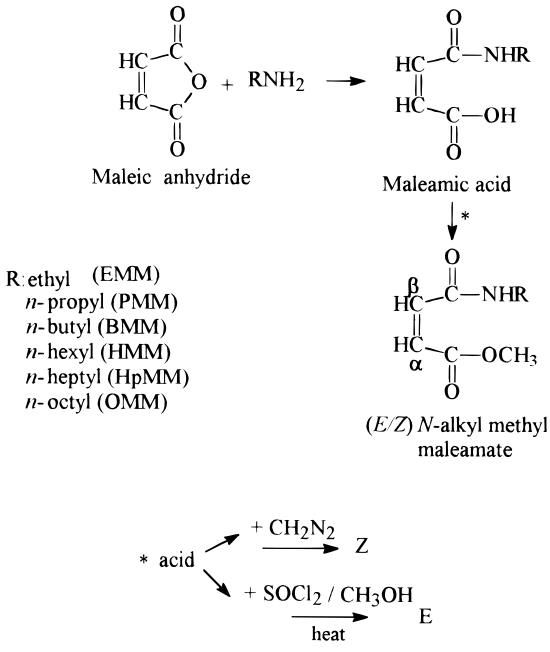

Fig. 1. Preferential pathways for the synthesis of (*E*) and (*Z*) *N*-substituted maleamates.

PC model took into account the presence of conjugated double bonds (*pi*-VESCF calculation).

2.7 Hydrophobic substituent parameter π

Maleamates are aliphatic molecules and the fragment system of calculating π was preferred.[13] The only variation in the molecules is at the *N*-alkyl chain, for each member of the series a $-CH_2-$ fragment being added from C2 to C8. The average log*P* increment of 0·54 per $-CH_2-$ was added, according to Hansch and Leo,[13] to the π value for $-CONHC_2H_5$ (−0·73).

3 RESULTS AND DISCUSSION

3.1 Synthesis and characterization of (*Z*) and (*E*) *N*-substituted methyl maleamates

As can be seen in Fig. 1, general methylation methods gave a mixture of (*Z*) and (*E*)-maleamates, but the formation of one or another can be favoured by varying the pH of the reaction or the temperature. It was important to work separately with each isomer since working with the mixture gave erratic biological data.

The production of the (*E*)-isomer from *N*-substituted methyl maleamates was favoured at acid pH and high temperatures, while the (*Z*) *N*-substituted methyl maleamate was obtained directly in neutral conditions.

In Table 1, [^{1}H]NMR chemical shifts of the doublet and multiplicity constants (*J*) for the double bond protons are given.

In Table 2 TLC R_f values for the (*Z*) and (*E*)-isomers are given. (*E*)-isomers always have higher R_f values than (*Z*)-isomers and their values increase with the length of the alkyl chain.

3.2 Suppressed food intake

Suppression of food intake on *T. infestans* nymph V for the synthesized *N*-substituted methyl maleamates quantified as ED_{50} is given in Table 3. Comparing the series of (*Z*) maleamates with the (*E*) *N*-hexyl and *N*-octyl methyl maleamates, shows that all the (*Z*)-isomers were effective, the ED_{50} value decreasing with the length of the alkyl chain, while the (*E*)-isomers were not effective at all. In other words, the longer the alkyl chain, up to C8, the higher the suppression of food intake observed for the (*Z*) *N*-substituted methyl maleamates.

TABLE 2
TLC for (*Z*) and (*E*)-isomers

Compound	*Isomer*	R_f^a
N-ethyl methyl maleamate (EMM)	*Z*	0·26
	E	0·31
N-propyl methyl maleamate (PMM)	*Z*	0·31
	E	0·38
N-butyl methyl maleamate (BMM)	*Z*	0·33
	E	0·39
N-hexyl methyl maleamate (HMM)	*Z*	0·33
	E	0·44
N-heptyl methyl maleamate (HpMM)	*Z*	0·37
	E	0·44
N-octyl methyl maleamate (OMM)	*Z*	0·39
	E	0·45

[a] Data are the means of two independent determinations.

TABLE 3
Suppression of Food Intake by *N*-substituted Methyl Maleamates on *T. infestans* Nymph V

Compound	ED_{50} *μg per insect*	*Confidence intervals*
(*Z*) EMM	16·4	4·9–54·1
(*Z*) PMM	8·6	3·2–22·7
(*Z*) BMM	6·6	3·9–11·2
(*Z*) HMM	5·0	2·7–8·8
(*Z*) HpMM	3·0	1·4–6·3
(*Z*) OMM	1·9	1·5–2·5
(*E*) HMM	>50·0	
(*E*) OMM	>50·0	

3.3 Reaction with GSH

Taking GSH as a molecular target of suppressed food intake for the synthesized *N*-substituted methyl maleamates, the reaction rates with GSH were measured *in vitro* by UV spectrometry in dioxane-buffer solutions at the λ_{max} obtained for each compound (Table 4). As the reaction rate depends on the substrate concentration, it is more convenient to use the initial reaction rate to compare the behaviour of the synthesized *N*-substituted methyl maleamates. From Fig. 2, the initial reaction rates of the (*Z*) *N*-substituted methyl maleamates with GSH were calculated (Table 4). Table 4 also shows the lack of reactivity of the (*E*) *N*-hexyl and (*E*) *N*-octyl methyl maleamates with GSH. After 5 h reaction time, there was no measurable absorbance. In Table 4, values for the density of charge on *β*-C are also given.

The initial reaction rates of (*Z*)-isomers with GSH decrease with increasing length of the alkyl chain up to C6, which correlates with the electron charge density on the *β*C of the double bond C=C (Table 4). For longer alkyl chains, charge density does not change any more and the initial reaction rates become similar.

(*E*) *N*-hexyl and *N*-octyl methyl maleamates do not react with GSH in the assay conditions (60°C). This fact could explain the lack of effect on food intake observed for these compounds and the variability of biological data when using *Z*/*E* mixtures.

TABLE 4
Initial Reaction Rates of *N*-substituted Methyl Maleamates with GSH

Compound	λ_{max} *(nm)*	*V (mM/min)*[a]	*Density charge on βC*
(*Z*) EMM	245·2	0·0095	0·069
(*Z*) PMM	245·7	0·0086	0·068
(*Z*) BMM	244·0	0·0073	0·067
(*Z*) HMM	245·7	0·0055	0·063
(*Z*) OMM	246·9	0·0073	0·063
(*E*) HMM	252·0	<0·0005	0·068
(*E*) OMM	254·1	<0·0005	0·068

[a] Data are the means of two independent determinations.

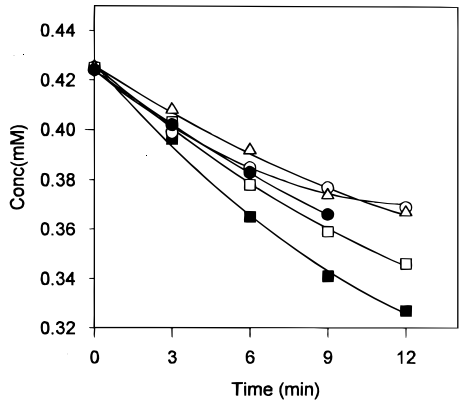

Fig. 2. Reaction with GSH curves for *N*-substituted maleamates 0·425 mM at pH 8·5 and 60°C (■) EMM, (○) PMM, (□) BMM, (△) HMM, (●) OMM.

3.4 Chemical structure–food intake suppression relationship

The effect on food intake of the synthesized (*Z*) *N*-substituted methyl maleamates, given by the corresponding ED_{50} values, was used as a measure for the biological activity in QSAR analysis, applying the Hansch approach.[14,15]

The physicochemical descriptors include the substituent parameter π for each (*Z*) compound, as a measure of the hydrophobic character of the molecule, and the initial reaction rate with GSH, as an indicator of chemoreception blockage.

Multiple regression analysis was used to correlate the physicochemical parameters with the food intake suppression. The following equation was obtained:

$$I/ED_{50} = 0.18\pi + 62.3V_{GSH} - 0.39$$
$$n = 5 \quad r = 0.99 \quad P = 0.005$$

The very good linear correlation found could be interpreted taking into account an initial distribution of the compounds on the epicuticle of the insect and a hydrophobic absorption, followed by a second step of attack to an —SH target, given by the reactivity with GSH.

The results obtained here represent a contribution to the search for new targets for the control of *T. infestans*, vector of Chagas' disease in our country.

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